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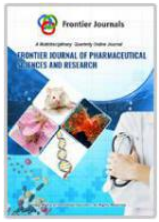

Original Research Paper

EVALUATION OF ANTIULCER ACTIVITY OF BERRIES AGAINST ETHANOL INDUCED GASTRIC ULCER

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ABSTRACT

Current therapies against *H. pylori* eradication involve the administration of proton pump inhibitors in combination with antibiotics; however, the development of resistant strains and poor compliance of patients have made the process of eradication complicated; furthermore the use of gastric acid suppressors in *H. pylori* therapies has shown to increases the risk of pneumonia and hip fractures. For all these reasons it is very important to find new strategies for the treatment of gastric diseases. Studies *in vitro* and *in vivo* demonstrate that the antioxidant and anti-inflammatory activities of some foods are due to their polyphenol content. Plant rich in tannins have a traditional use for treating gastric ulcer, and tannins showed anti-bacterial activity against *H. pylori*. The chemical composition of tannins depend on the fruit source, sanguiin H-6 and lambertianin C representing the main compounds in blackberry and raspberry. On this basis, the aim of this research was to clarify the anti-ulcer effect of the extracts enriched in tannins from berries at gastric level. Gastric damage was assessed in a blind manner by a scoring system based on the number and severity of the lesions: 0, no lesions; 1, lesions 1–2 mm; 2, lesions 2–3 mm; 3, lesions 3 mm. Hence it was observed that the number of lesions was reduced in the rats treated with the extract of wild berries showing its protective effect against ethanol induced ulcers.

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1. INTRODUCTION

The gastrointestinal tract represents an important barrier between the human hosts and microbial populations. One potential consequence of host-microbial interactions is the development of mucosal inflammation, which can lead to gastritis and ulcer. Gastritis and ulcers are very common inflammatory based diseases which can be caused by *H. pylori* infection, chemical factors or immunological disorders. *H. pylori* is the leading cause of gastritis, it colonizes the gastric mucosa of over 80% of human population in developing countries and at least 50% of the world's human population. Recently, this bacterium has been classified as type I carcinogen by the World Health Organization, its eradication in infected individuals significantly decreases the risk of developing gastric adenocarcinoma.[1]

Fruits and vegetables demonstrated to exert multiple biological effects on the mucosa of the gastrointestinal tract due to their antioxidant content and they play a crucial role in the maintaining of gastric mucosa homeostasis by counteracting potential damage exerted by ROS.[2]

Current therapies against *H. pylori* eradication involve the administration of proton pump inhibitors in combination with antibiotics; however, the development of resistant strains and poor compliance of patients have made the process of eradication complicated; furthermore the use of gastric acid suppressors in *H. pylori* therapies has shown to increases the risk of pneumonia and hip fractures. For all these reasons it is very important to find new strategies for the treatment of gastric diseases.[1] Studies *in vitro* and *in vivo* demonstrate that the antioxidant and anti-inflammatory activities of some foods are due to their polyphenols

content. Polyphenols provide protection against different diseases such as diabetes, obesity and stroke. Among the natural sources rich in polyphenols there are wild berries.[2] The main objective of the present study was to evaluate the anti ulcer activity of extract of wild berries in experimental animals against ethanol-induced gastric ulcer.

2.PLANT PROFILE

The term "wild berries" identifies some edible berries, with high organoleptic properties, distributed mainly in temperate areas and widely used both in cooking and in pharmacy. Fruits belonging to this group are for example blackberry, raspberry, commercial and wild strawberry, bilberry, cranberry, lingonberry, black currant and alpine currant.[3]

Chemical constituents

Berries hold an important position among the fruits for their highly antioxidant phytochemicals. Wild berries share, besides the organoleptic properties, the presence of two classes of molecules of particular interest for biological activities: flavonoids and tannins.[3]From the chemical point of view flavonoids are flavone derivatives (2-phenyl-γ- benzopyrone). Depending on the structure, flavonoids are divided in several sub-classes. Anthocyanins are flavonoids very common in berries.Tannins can be classified into two main groups: hydrolysable tannins and condensed tannins. Hydrolysable tannins can be divided in two subgroups: gallotannins, if they release mainly gallic acid during hydrolysis, and ellagitannins, if they release mainly ellagic acid (EA). The chemical composition of tannins depend on the fruit source, sanguiin H-6 and lambertianin C representing the main compounds in blackberry and raspberry. [3]

Biological uses

The beneficial effects on human health related to consumption of berries include the prevention of inflammation, oxidative stress, cardiovascular disease, cancers, diabetes mellitus type 2, obesity and neurodegeneration.[3]

3. MATERIAL AND METHODS

Berries were collected from local market and maintained at -22°C. Fruits were extracted with a mixture acetone/water (70/30 v/v); the ratio fruit/solvent was 60 g/250 mL. Berries were homogenized with an 847-86 model Osterizer blender and centrifuged. Polyphenol-rich extracts were evaporated until dryness in a pear-shaped flask, using rotary evaporation under reduced pressure at 37 °C. The sample was diluted to 1 L with mixture methanol/water (30/70 v/v) and filtered using a Durapore 0.45 mm filter. The purification was carried out using an established method with minor changes due to the high volume of the samples.[3]

Briefly, a column cartridge (1064 cm), connected to a vacuum line, was packed with Sephadex LH-20 resin, pre-washed with 50 mL methanol and then equilibrated with 100 ml methanol/water (30/70 v/v).[3] The aqueous methanol extract (50 mL) was loaded and polyphenols, such as anthocyanins, were washed off with 500 mL methanol/water (30/70 v/v). The fraction containing the ellagitannins was eluted using 350 mL acetone/water (70/30 v/v).[4] The tannin-rich extracts (TEs) were dried using rotary evaporation under reduced pressure at 37°C and reconstituted in 5 ml methanol, added to 350 ml diethyl ether and precipitated with hexane (700 ml). The TE fraction was recovered by filtration and dried.[4]

4.EXPERIMENTAL SETUP

Animals

Twenty Four Wistar rats, weighing 95–120 g, were used. 3 Rats per cage were housed under constant environmental conditions (22 ± 1°C, 50 ± 5% relative humidity, 12-h light/12-h dark cycle), with free access to standard laboratory rat chow and tap water. Animals were acclimatized for a period of at least 7 days before the use. The study was approved by the Institutional Animal Ethics Committee of Anwar Ul Uloom college of Pharmacy, Hyderabad. All efforts were made to minimize animal suffering.[6]

Before the experiment, the animals were randomly divided in 4 groups (6 rats in each group) and treated intragastrically (i.g.) by gavage. The dose of TEs was calculated on the basis of a daily consumption of 125 g of fresh fruit by a human healthy adult of 70 kg. The day before the induction of gastric lesions, rats were placed in individual metabolic cages and deprived of food, with free access to tap water for 20 hours. The last administration of TEs extracts, quercetin (as positive control) or vehicle was given 120 minutes before ethanol treatment.[6]

Experimental Setup

The *in vivo* study aimed to demonstrate the protective effects was carried out on 24 Wistar rats, divided into 4 groups according to the different type of treatment, as follows:

Group A: animals pre-treated for 10 days with only vehicle (10% polyethylene glycol 400) without administration of pure ethanol.

Group B: animals pre-treated for 10 days with only vehicle (10% polyethylene glycol 400) followed by administration of pure ethanol for 1 hour.

Group C: pre-treatment for 10 days with 100 mg/kg/day of quercetin dissolved in 10% polyethylene glycol 400, followed by administration of pure ethanol for 1 hour.

Group D: pre-treatment for 10 days with 200 mg/kg/day of TE-black dissolved in 10% polyethylene glycol 400, followed by administration of pure ethanol for 1 hour.[7]

Assessment of gastric mucosal damage

One hour after the administration of 1 mL of ethanol, rats were sacrificed under ether anesthesia by cervical dislocation; the stomach was removed and opened along the greater curvature. The stomach was rinsed with water, pinned open for microscopic examination by a microscope.. Gastric hemorrhagic lesions in the glandular part were examined under a dissecting microscope (X10). Gastric damage was assessed ina blind manner. The Ulcer Index (UI) was obtained by a 0–3 scoring system based on the number and severity of the lesions. Severity was defined according to the length of the lesions: 0, no lesions; 1, lesions 1–2 mm; 2, lesions 2–3 mm; 3, lesions >3 mm. UI was calculated as the total number of lesions multiplied by their respective severity score.[8]

Preparation of gastric mucosa homogenates

Samples of 50 mg from normal and ulcerated rat gastric mucosa were homogenized in buffer A [10 mM TRIS-HCl (pH 8), 150 mM NaCl, 1

mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 µg/ml aprotinin, 2 µg/ml leupeptin, and 1% Triton X-100] using Tissue Lyser II (Qiagen) for 2 minutes at the highest frequency 30/s. The homogenates were centrifuged at 12,000 g for 10 min at 4°C and the supernatants collected, and stored at -80°C until use. Protein concentration was determined using Bradford protein assay (Bio-Rad) with bovine serum albumin (Sigma-Aldrich,) as a standard with emission and excitation wavelengths of 528 and 485 nm, respectively, by using a microplate fluorescence reader. The ORAC values were calculated as area under the curve and expressed as micromole of Trolox equivalent (TE) per gram of gastric mucosa sample.[8]

Statistical analysis

All data are expressed as mean ± s.d., with the exception of the *in vivo* experiments expressed as mean ± s.e.. Differences between means were calculated using the unpaired t test or one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple group comparisons. Statistical analysis was done using GraphPad Prism $p<0.05$ was considered statistically significant.[9]

5. RESULTS AND DISCUSSION

TABLE 1 Phytochemical screening of the aqueous extract

CONSTITUENT	RESULT
Alkaloids	+
Saponins	+
Taninns	+
Phytosterols	+
Phenolics	+
Volatile oils	-
Terpinoids	+
Flavonoids	+
Glycosides	+
Absent: -	Present: +

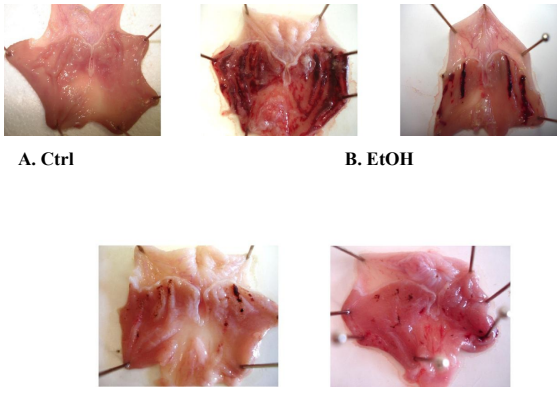
The quantification of tannins present in the extracts was performed by UPLC-PDA-MS. To determine the content of the main compounds, the analysis was performed at 260 nm according to the protocol. In enriched fractions, tannins from blackberry (TE-black) corresponded to 343 mg/100 g of fresh fruits, while tannins from raspberry (TE-rasp) were 155 mg/100 g. The composition of TEs was as follows: in TE-black sanguiin H-6 represented 12%, lambertianin C 56%, and ellagic acid 1% of the precipitate, while in TE-Rasp sanguiin H-6 represented 19%, lambertianin C 35%, and ellagic acid 1%. Sanguiin H-6 and lambertianin C, compounds belonging to the class of ellagitannins, account for more than 50% of both the extracts.[3]

TABLE 2 Effect of the treatment with the extracts on rat weight

Group	Initial weight (g)	Final weight (g)
A	185.3 ± 6.7	214.8 ± 4.9
B	191.4 ± 5.3	221.3 ± 7.9
C	180.6 ± 8,2	211.7 ± 6.5
D	183.0 ±7.4	215.3 ± 5.3

TABLE 3 Ulcer Index measured in all animal groups

INDEX	(A) Vehicle - Ethanol	(B) Vehicle + Ethanol	(C) Quercetin + Ethanol	(D) TE-black + Ethanol
Animal 1	0.0	16	4.0	5.0
Animal 2	0.5	19	1.5	7.0
Animal 3	0.0	20	1.0	2.5
Animal 4	0.0	18	3.5	2.0
Animal 5	0,0	15	1.0	4.0
Animal 6	0.5	19	1.5	6.0
Mean	0.2	17.8	2.1	4.4
Standard error	0.1	0.8	0.5	0.8



A. Ctrl **B. EtOH**

C. Quercetin **D. TE-black 200 mg/kg/day**

FIGURE 1 Protective effect of TEs from blackberry and raspberry against ethanol induced gastric injury.

Gastric damage was assessed in a blind manner by a scoring system based on the number and severity of the lesions: 0, no lesions; 1, lesions 1–2 mm; 2, lesions 2–3 mm; 3, lesions 3 mm. Ulcer Index was calculated as the total number of lesions multiplied by their respective severity score.[9]

6. CONCLUSION

In recent years, the study of medicinal and edible plants able to treat or

prevent the development of various chronic diseases is attracting more and more interest. It has been demonstrated that fruits and vegetables occurring in the human diet, including berries, may exert a variety of health benefits mainly due to their antioxidants content. The anti-oxidant and anti-inflammatory activity of these fruits can be attributed to their polyphenols content. Several works in the literature suggested the importance of the consumption of products rich in polyphenols in relation to gastritis induced by *H. pylori*. Recent studies have established that the intake of berries has a positive effect on human health, and this ability has been ascribed to the high phenolic content. Among polyphenols, in the last few years anthocyanins and, to a lesser extent, condensed tannins, received more attention, whereas the biological effects of ellagitannins have been poorly investigated. Plant rich in tannins have a traditional use for treating gastric ulcer, and tannins showed anti-bacterial activity against *H. pylori*. The chemical composition of tannins depend on the fruit source, sanguiin H-6 and lambertianin C representing the main compounds in blackberry and raspberry. Different studies have demonstrated that tannins are stable at the physiological conditions of the stomach: gastric pH, between 1.8 and 2.0, and digestive enzymes are not able to hydrolyse or metabolize this class of molecules, and they are not absorbed in this district. Metabolism of tannins takes place in the intestine where the physiological pH of the small intestine causes the hydrolysis of ellagitannins and the release of ellagic acid.[3-6]

Fruits and vegetables demonstrated to exert multiple biological effects on the mucosa of the gastrointestinal tract due to their antioxidant content and they play a crucial role in the maintaining of gastric mucosa homeostasis by counteracting potential damage exerted by ROS. At this regard, among fruits, wild berries possess phytochemical contents with high antioxidant activity. Berries contain two classes of molecules of particular interest for biological activities: flavonoids and tannins. Plants rich in tannins have a traditional use for treating gastric ulcer and tannins showed anti-bacterial activity against *H. pylori*.[3]

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7.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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